

The culture of penaeid shrimp as a viable industry has been established in Japan for over a decade, and in recent years has experienced marginal success in other areas of the world. Where it exists, the present industry depends on obtaining spawners from wild stock to provide larval animals for production facilities. This procurement of wild spawners is costly and does not lead to the development of a domestic stock, a prerequisite for the optimum development of an animal production related industry.

Successful reproduction of penaeid shrimp in captivity has been accomplished at several laboratories, but the refined techniques necessary to produce animals when they are needed and in the numbers necessary to support a commercial operation have yet to be developed. Johnson and Fielding (1956) using ponds with limited water exchange reported reproduction of Penaeus setiferus in Florida. Moore, Sherry and Montanez (1974) using flow-through raceway systems with seven exchanges of water per day reported reproduction of P. californiensis in Puerto Penasco, Mexico; and Griessinger et al. (1975), in Tahiti, reported reproduction of P. merguensis, P. semisulcatus, P. japonicus, P. aztecus and Metapenaeus ensis using flow-through tanks. In the case of P. aztecus unilateral eyestalk ablation was necessary for female gonadal development.

The species used in the present study were P. stylirostris and P. setiferus. The first species is nonindigenous to the Texas Gulf Coast while the second is a common animal to this area. Legal aspects of our culture permits prevent the utilization of flow-through systems with nonindigenous species in a reproductive condition; therefore, the purpose of this study was to investigate the practicality of using closed-pond systems to produce a breeding stock of nonindigenous penaeid shrimp.

METHOD AND MATERIALS

In May, 1974, postlarval P. stylirostris were stocked at 112,500/ha in a 0.2 ha pond in Corpus Christi, Texas. At the end of 139 days shrimp averaging 150.0 mm in length and 30.0 g in weight were harvested. Ovarian development was not observed in the females, but the males exhibited small developing spermatophores. Five hundred of these shrimp were transported to the National Marine Fisheries Laboratory in Galveston, Texas and overwintered in a heated raceway. The raceway is a closed system 3.0 m wide x 24.0 m long x 0.9 m deep and provided with two pressurized anthrofelt plate filters (Mock, Neal and Salser, 1973). Water was added periodically to compensate for water lost due to evaporation and leakage. The shrimp were fed frozen squid (3% body weight/day) and the water was monitored for temperature, salinity and pH (Table 1).

In April, 1975, 100 shrimp were returned to Corpus Christi and along with 17 adult P. setiferus captured offshore, were stocked in a 0.1 ha clay bottom pond. The maturation pond was one of a series of 18 0.1 ha ponds located adjacent to the Barney M. Davis Generating Station's 1100-acre cooling lake (Conte, 1975). A water depth of 41 cm at the upper end and 92 cm at the lower end was maintained in the pond. The pond was kept in a static condition with no flow-through of water. The shrimp were fed Ralston Purina Marine Ration 20, (1.1 kg per .1 ha/day), and the water was monitored for temperature, salinity, pH, and oxygen. Plankton samples taken from the pond were monitored throughout the study for the presence of penaeid eggs and larvae.

Mature male and female P. stylirostris were confined in pyramid-shaped soft net cages with a 1.0 m base and placed in the deep end of the pond. The shrimp were checked periodically for evidence of gonadal development and copulation. Copulation was defined as the transfer of a spermatophore.

Two unilateral eyestalk ablation experiments were conducted on a total of 12 female P. stylirostris. In each test 6 females exhibiting no ovarian development were removed from the pond, ablated and confined in a soft net cage (1.5 m wide by 2.75 m long by 0.75 m deep) along with 6 unablated control females showing no ovarian development, and 6 male shrimp exhibiting developed spermatophores.

RESULTS AND DISCUSSION

Uncaged animals: In late June, 1975, plankton samples were taken from the maturation pond and examined for evidence of penaeid eggs or larvae, neither of which were found. The pond was harvested and 47 male and 45 female shrimp were examined, measured and returned to an adjacent pond. All males of both species had well developed spermatophores and remained in this condition throughout the study. The females averaged approximately 52.0 g in weight and 190.0 mm in length. Eleven of the female P. stylirostris and one P. setiferus were in various stages of ovarian development, but there was no evidence of copulation.

From early July through mid-September the maturation pond was periodically seined with a 50.0 m net. With each pull, we were able to examine approximately 75% of the females in the pond. From July through September P. stylirostris exhibited all stages of gonadal development. Only one P. setiferus was found with developing ovaries. At any examination the number of female P. stylirostris with gonadal development ranged from 15 to 50%. It soon became apparent that handling the unfertilized females caused reabsorption of the ovaries. Whenever the pond was seined within 24 hours of a previous seining, the second attempt would produce fewer numbers of females exhibiting ovarian development.

Seining the pond during daylight produced only one shrimp with evidence of copulation. This was a female *P. stylirostris* with the remnant of an attached spermatophore. As we were unprepared, no attempt was made to spawn the animal. Six other *P. stylirostris* with attached spermatophores were captured, all between 2200 and 2400 hours.

Animals with attached spermatophores were successfully spawned in 18.9 liter carboys containing pond water passed through a 5 μ filter. The water was maintained at 28°C, was aerated and contained ethylenediamine tetra-acetic acid (0.2035 g/18.9 L) and erythromycin (50 mg). Unfortunately embryonic development was incomplete and hatching was never observed. The first three spawns produced zygotes which never developed beyond the 8-cell stage. Microscopic examination revealed particulate material, apparently clay, adhering to the hatching coat of the retarded embryos.

Assuming that the presence of this particulate material might have been responsible for the retarded development, greater care was taken in the filtration of water for the next spawning series. The fourth spawner was placed in water which was filtered as described above and in addition recirculated through a diatomaceous earth filter. This spawner produced zygotes which developed to the morula stage (32 to 64 cell) before arrestment. Microscopic examination offered no explanation for the arrestment; the embryos and their investment coats appeared normal.

Investigators have reported poor larval rearing success in water with a salinity above 36 ppt (Harvey Persyn, personal communication; Cornelius Mock, personal communication). We had been using water with a salinity of 42 ppt and felt this might have been responsible for the embryonic arrestment. As a result, our fifth and sixth spawners were placed in filtered pond water with a salinity of 35 ppt. The spawned eggs continued development to the morula stage then stopped. It must be remembered that the females received an abrupt salinity change from 42 to 35 ppt just prior to spawning. Whether this change affected development is pure conjecture at this point. A series of experiments are underway to determine the effect of salinity variations on embryonic development.

From the number of females exhibiting gonadal development, and the sexual activity observed, it is probable that numerous spawnings occurred in the pond. Plankton samples taken from the pond revealed a massive population of copepods, chaetognaths, and other zooplankton, but no evidence of penaeid eggs or larvae was noted. Salinity ranged from 41.0-50.0 ppt (Tables 2 and 3) and this, along with the large grazing population of zooplankton, would be detrimental to the survival of penaeid eggs and larvae.

Caged animals: Male and female *P. stylirostris* were placed in pyramid-shaped cages which sat on the pond bottom and were moved periodically to allow access to benthic organisms and supplementary ration. The caged females exhibited no ovarian development, even

during August and September when maximum sexual development was observed in unconfined females. Evidence of copulation was not observed even though the caged males possessed and retained well developed spermatophores. Within 10 days both caged males and females became soft with the subsequent development of chitinous infections on their abdomens, telsons, uropods, and pleopods, a condition not observed in unconfined animals. The animals usually died within 18 days enclosure. The cage experiments were repeated several times with the same results. An explanation for the poor survival of these animals is in question, though the abrasive effects of the net leading to chitinous infection is suspected to be the cause.

Three experiments were conducted where unconfined females exhibiting signs of gonadal development were transferred to cages containing males exhibiting developed spermatophores. These females reabsorbed their ovaries within two days and no copulation occurred. There was no further development of ovarian tissue and in time the condition of both male and female shrimp would deteriorate as described earlier.

In the unilateral eyestalk ablation tests, 5 of the ablated females in the first test and 4 ablated females in the second test survived, and of these, all exhibited ovarian development within 4 days. The unablated females showed no signs of developing ovarian tissue and no evidence of copulation was observed in test or control animals. In each experiment, mortality of the ablated animals reach 100% by 7 days; however, no mortality was observed in the unablated female or male shrimp. The remaining male and female shrimp were retained in the cage for further observation. When it became apparent that no sexual development was occurring in the females and the general condition of all the animals was deteriorating, the experiment was terminated and the animals released into the pond. The rapid ovarian development observed and degree of mortality in the ablated females corresponds with the results of Duronslet et al. (1975).

SUMMARY

This work represents initial attempts to determine the feasibility of closed-pond systems for the maturation and breeding of nonindigenous species of penaeid shrimp. While the results presented here are only preliminary, several significant points have become apparent. (1) After rearing *P. stylirostris* in pond systems in Corpus Christi for three years, only animals which were previously overwintered and in their second season exhibited maturation and breeding. (2) Pond-maintained *P. stylirostris* in this study appear to breed after 2200 hrs. and spawn within 6 hours. (3) Methods of recovering shrimp must be refined since the disturbance of females promotes ovarian reabsorption. (4) To anticipate recovery of larvae

hatched in pond systems appears doubtful. (5) Shrimp did not tolerate total enclosure in soft net cages. (6) Shrimp that have undergone unilateral eyestalk ablation and then placed in net cages in the pond suffered extreme mortalities.

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Table 1. Raceway: Hydrological data from 15 October 1974 through 24 April 1975.

		Range	Mean
Temperature	C	22.0-30.0	27.0
Salinity	ppt	26.0-32.0	30.0
pH		7.8-9.2	8.8

Table 2. Pond: Hydrological data from 24 April 1975 through 12 September 1975.

		Range	Mean
Temperature	C	26.9-31.5	28.6
Salinity	ppt	38.0-50.0	44.3
Oxygen	ppm	1.0-8.6	3.5
pH		7.7-8.8	8.4

Table 3. Pond: Hydrological data during the period of maximum sexual development and activity from 5 August through 12 September 1975.

		<u>Range</u>	<u>Mean</u>
Temperature	C	27.6-31.5	28.9
Salinity	ppt	39.0-50.0	44.2
Oxygen	ppm	1.0-5.6	2.4
pH		8.0-8.6	8.3